fungus ages the hyphæ are longer and fewer conidia remain in chains. When the conidia are not so profuse as to cover the surface of the spots, with the aid of a lens the hyphæ can be seen in definite clusters. As the hyphæ age it is not infrequent for the conidia to be unilateral when the hypha will be curved as some are represented in fig. 4.

The figures will be easily understood, the progress of development of the hyphæ being shown from figs. 1 to 3.

Ramularia areola n. sp. Spots hypophyllous, rarely amphigenous, pale at first, becoming darker, 1–10 mm. (mostly 3–4 mm.), angular, irregular in shape, limited by the veins of the leaf, conidia in profusion giving a frosted appearance to the spots. Hyphæ hypophyllous, rarely amphigenous, fasciculate, in small clusters distributed over the spots, subnodose, older ones frequently branched below, more rarely above where they are toothed, teeth frequently unilateral when the hyphæ are curved instead of zigzag, several times septate, stouter below, hyaline, 25–75μ × 4·5–7μ. Conidia oblong, usually abruptly pointed at the ends, sometimes rounded, 1 to 3-septate, concatenate in the early development of the hyphæ, hyaline, 14–30μ × 4–5μ.—On leaves of Gossypium herbaceum, Auburn, Alabama, Oct.–Nov. 1889. Geo. F. Atkinson.

Auburn, Ala.

Notes on technique. I.

JAMES ELLIS HUMPHREY.

Although the value and importance of good permanent mounts for the microscopic study of algæ and fungi is generally recognized, most of the methods by which they are prepared are tedious in detail or unsatisfactory in results, or both.

The balsam slide is to be regarded as the one embodying the desirable features of such a preparation ; namely, transparency, simplicity in manipulation and indefinite preservation of the specimen in a solid medium which will neither run nor leak. Balsam, however, is unsuitable as a medium for most of the thallophytes, and we are compelled to seek a substitute which is less dense and which mixes readily with water. The best results have hitherto been obtained with the algæ by the use of solutions

of preservative substances in water, and with the fungi by the use of glycerine, usually diluted with some antiseptic fluid or solution of low density. But all such media are permanently fluid and require the use of artificial cells to contain them. These cells when not built wholly of some cement depend on such a material for their security, and require to be made with the greatest care and to be freshly coated at intervals. After a considerable experience with nearly all the best cements, I am convinced that none is wholly to be relied on, while the trouble of manipulating them and of preparing satisfactory mounts in fluid makes the abandonment of the whole technique extremely desirable. Further, very few of the liquids in use preserve delicate histological features or differential staining.

About the only available non-resinous solid medium is *glycerine jelly* which has proved so entirely applicable and so well adapted to the preservation of algæ and fungi that fluid media and cements, which have been used chiefly for these plants, may be now relegated to the limbo of superseded evils.

The object of the present note is to call the attention of American botanists to a mode of treatment preliminary to mounting in glycerine jelly, which is not complicated in detail, is very satisfactory in its results, and is widely applicable among the thallophytes. In *Hedwigia* for 1888, Heft 5 and 6, page 121, Dr. L. Klein described his method of preparing slides of fresh-water algæ, which the writer has found very successful and convenient.

The prime secret of success in the use of glycerine jelly is to have the object thoroughly permeated by glycerine before it is placed in the medium. But the well-known dehydrating action of glycerine causes a rapid and irreparable shrinking and collapse of delicate watery tissues placed in it, even though it be very much diluted. Several reagents extensively used by histologists have the effect of hardening or coagulating tissues, so that they become much less easily distorted by subsequent manipulation. Of these reagents there is one which acts very quickly and produces hardly any perceptible change in the living appearance of the tissue or organism, simply fixing and preserving the details of its structure. This is the substance known as *osmic* or *perosmic acid*. It is used in aqueous solution of a strength not exceeding one per cent. of the acid. Experience has shown that

this reagent is especially adapted to fixing and hardening the more delicate algæ without structural change. The plants to be treated are placed in a drop of water on a slide, and, if they are comparatively large, a drop of the one per cent. solution of osmic acid is added and the whole is allowed to stand for perhaps half an hour. The fluid must, however, be drained away from the plants before they begin to show the browning or blackening which results from the prolonged action of the acid. In the case of very small or unicellular plants, it is sufficient to invert the drop of water containing them over the mouth of the reagent bottle, since the fumes of the acid will produce the desired effect in a short time.

Even after being thus hardened, algæ can not safely be treated with glycerine of greater strength than that resulting from a mixture of one part with eight or ten parts of water. They should be allowed to stand, protected from dust, in a small quanity of this mixture, which gradually decreases in volume and increases in density until it nearly reaches that of pure glycerine, by the spontaneous evaporation of its water. The concentration is so slow that not the slightest shrinking ordinarily occurs in plants first fixed with osmic acid. A couple of days is usually required for its accomplishment.

After draining away the excess of glycerine, one may add a drop of glycerine jelly, warmed just sufficiently to render it fluid, and then cover at once. Air bubbles are more easily avoided if both slide and cover glass are gently warmed. Slides carefully prepared by this method preserve details of structure and the natural appearance of the plants with striking completeness and show no change after a considerable time, as the writer can testify.

Besides applying this process to the preservation of freshwater algæ, for which it was especially recommended by Dr. Klein, I have had opportunity to test its applicability to other thallophytes. At the marine biological laboratory at Wood's Holl, Mass., in August last, it was used by the students in botany in preparing all their slides of marine algæ, and with much success. Members of all the chief groups were mounted with excellent results. Some of my most careful students report that all, or most of their preparations have remained unchanged in every respect, and they were certainly, when fresh, the best I have ever seen. The algal pigments are, save in a few exceptional species, not at all altered.

The action of osmic acid does not seem to sufficiently harden the walls and cell-contents of the most delicate Florideæ (Callithamnion, Griffithsia, fringing hairs of Spyridias, Dasya, etc.) to prevent their shrinking even in very dilute glycerine, but no one of many other reagents experimented with gave much better results. Perhaps some of the workers at Wood's Holl, the present season, can remove the difficulty.

Most fungi suffer no change in dilute glycerine, although not previously hardened, and they may be well preserved in glycerine jelly. Such as are too delicate to do so otherwise may be enabled, in most cases, to withstand the distorting influence of glycerine by hardening in osmic acid, as described for the algæ. I have not yet succeeded, however, in satisfactorily preserving Saprolegniaceæ in this way, though the most delicate Mucoraceæ and Hyphomycetes do finely.

In short, it is not too much to say that the way is opened, by the process above described, toward the abandonment of fluids and cements and all the bothersome manipulation connected with their use, and the substitution of a technique simpler in detail and far more satisfactory in results.

Amherst, Mass.

On the nature of certain plant diseases.

ALEXANDER LIVINGSTON KEAN.

De Bary in his paper "On some Sclerotiniæ and sclerotium diseases,"[1] published in 1886, was the first to show that Sclerotinia (Peziza) sclerotiorum while apparently growing as a parasite actually grows as a saprophyte, but gives off in the process of its growth a ferment which swells the cell-walls and kills the tissue of the host, thus preparing the way for the fungus. In 1888 Marshall-Ward described a Botrytis growing upon a Lilium candidum which behaves in the same manner.[2] De Bary found that liquid obtained from vegetable tissue infested with Sclerotinia was capable of producing the characteristic decomposition of pieces of healthy tissue placed in it. Marshall-Ward not only obtained this same result, but also observed under the microscope drops of

[1] Bot. Zeit. 1886, nos. 22, 23.

[2] Annals of Botany, Nov., 1888. Vol. ii, no. vii.